PROC. BIOL. SOC. WASH.
107(2), 1994, pp. 391–397

ADVERTISEMENT CALLS AND RELATIONSHIPS OF CHILEAN FROGS *EUPSOPHUS CONTULMOENSIS* AND *E. INSULARIS* (AMPHIBIA: ANURA: LEPTODACTYLIDAE)

J. Ramón Formas and Lila Brieva

Abstract.—Advertisement calls of *E. contulmoensis* and *E. insularis* are described. The calls of both species consist of a single short note. Call data, together with chromosomal information, indicate that *E. contulmoensis,* and *E. insularis, E. roseus, E. calcaratus* and *E. migueli* comprise a related group distinct from *E. vertebralis* and *E. emiliopugini.*

Calls of frogs can be useful in revealing evolutionary relationships at the species level. In general, closely related species have some acoustic characteristics in common (Blair 1958, Barrio 1965, Mecham 1971, Martin 1972, Schiøtz 1973, Kuramoto 1974, Ryan 1988, Straughan & Heyer 1971). In order to establish evolutionary trends related to the diversification of the genus *Eupsophus,* we analyzed the advertisement calls of *E. contulmoensis* and *E. insularis.* Both calls are compared with those other members of the genus that were previously described by Formas & Vera (1980), Formas (1985, 1989), and Penna & Veloso (1990).

Eupsophus is the most speciose taxon of the rather reduced anuran fauna of the *Nothofagus* forests of southern Chile and Argentina. Seven species have been described: *E. roseus, E. calcaratus, E. migueli, E. contulmoensis, E. insularis, E. vertebralis* and *E. emiliopugini.*

E. contulmoensis has been collected in the type locality of Contulmo (37°52′S, 73°12′W), Nahuelbuta Range (Ortiz et al. 1989), and *E. insularis* is endemic to Mocha Island (38°22′S, 73°56′S; 38 km W of the coast of Arauco Province) (Formas & Vera 1982).

Methods and Materials

Field recordings were made at 19 cm/sec on an Uher 4000 Report-IC portable tape-recorder and an Uher 517 microphone. Call durations, number of notes per call, and note duration were analyzed with a storage oscilloscope Tektronix 5113. Audiospectrograms were made with a Kay Elemetric audiospectrograph model 675, using 85–8000 Hz frequency scale and narrow (45 Hz) band filter. Call repetition rates were measured in the field by counting consecutive calls over one minute. Water temperature, location and behavior of the individuals used in the analysis were taken at the time of recording. Specimens and tapes were deposited in the collection of Amphibians of the Institute of Zoology, Universidad Austral de Chile (IZUA), Valdivia.

Eupsophus contulmoensis Ortiz, Ibarra-Vidal, & Formas, 1989

The advertisement call of *E. contulmoensis* was recorded at the type locality (Natural Monument of Contulmo, Malleco Province). Description is based on 25 calls from seven specimens recorded at 11.8°C (water temperature) during November 1990. Males

Table 1.—Call characteristic (mean and range) of *Eupsophus* species (S, short call; L, long call)

Species	Call type	Notes per call	Call length sec	Repetition rate	Dominant frequency (Hz)
E. contulmoensis		1	0.18 (0.15–0.20)	23.3 (15–34)	(1100–2000)
E. insularis		1	0.16 (0.14–0.18)	7.8 (4–12)	(1500–2100)
E. roseus †	S	1	0.20 (0.19–0.21)	64.0 (60–72)	(1600–2900)
**	S	1	0.10 (0.07–0.16)	25.1 (11.1–60)	(1250–1350)
**	L	32.1 (8–47)	2.73 (0.65–40.0)	10.2 (93–112)	(1220–1470)
E. migueli ††	S	1	0.24 (0.20–0.35)	6.0 (3–8)	(1500–2500)
**	S	1	0.20 (0.16–0.26)	4.2 (2.4–6.6)	(1170–1820)
††	L	24.0 (19–33)	3.40 (3.30–4.40)	6.0 (5–8)	(900–1500)
**	L	12.3 (4–23)	1.07 (0.30–2.16)	5.4 (2.4–8.4)	(1210–2000)
E. calcaratus ††		1	0.19 (0.15–0.21)	19.0 (16–25)	(1100–2700)
E. vertebralis †		5.0 (4–6)	0.60 (0.40–0.80)	4.0 (2–10)	(1100–2500)
**		5.6 (3.8)	0.64 (0.40–0.88)	27.8 (18.6–36.6)	(700–1110)
E. emiliopugini *		2	0.50	—	(729–1320)

† From Formas & Vera (1980); †† Formas (1985); * Formas (1989); ** Penna & Veloso (1990).

were observed and collected while they were calling from cavities in the ground at the border of a stream close to the forest. Individuals were organized into duets and trios spaced apart by 40–180 cm. Generally males called in alternation. The advertisement call of *E. contulmoensis* is composed of only one note (Fig. 1a, d; Table 1) that lasts 0.15–0.18 seconds. The dominant frequency is spread between 1100–2000 Hz and the repetition rate is 15–34 calls/minute. All calls showed modulation and defined harmonics are present at about 500 Hz intervals.

Eupsophus insularis (Philippi)

Description is based on 18 calls recorded from three individuals at Isla Mocha, during December 1989. Calls were recorded at a water temperature of 12°C. Males were observed and captured while they were calling from cavities in the ground close to a stream at the border of the forest. The individuals call isolated and neither duets nor trios were detected. Calls consist of a single note (Fig. 1b, e; Table 1) lasting 0.14–0.18 seconds. The call is modulated and defined harmonics are present at about 500 Hz intervals. Maximum energy is spread over a frequency range of 1500–2100 Hz.

E. roseus (Dumeril & Bibron), *E. migueli* Formas, *E. calcaratus* (Günther), *E. vertebralis* Grandison, and *E. emiliopugini* Formas

Call characteristics of the above cited species were described previously (Formas & Vera 1980; Formas 1985, 1989; Penna & Veloso 1990) and are depicted in Table 1.

Discussion

It is noteworthy that all *Eupsophus* species call during the spring-time from cavities along margins of small streams in the forest (Formas & Vera 1980; Formas 1985, 1989; Penna & Veloso 1990). Among the anuran fauna of the temperate *Nothofagus* forest of South America, males of *Eupsophus* species are unique in calling from flooded underground cavities near streams.

Based only on temporal parameters (call length and notes per call) of advertisement calls, Formas (1985) established two clusters of species within the genus *Eupsophus*. The first group contains *E. roseus, E. migueli* and *e. calcaratus,* whose calls consist of only one note lasting 0.10 to 0.20 seconds (Table 1). Calls of *E. contulmoensis* and *E. insularis* (a single note lasting 0.14 to 0.18 seconds) provide good evidence for group-

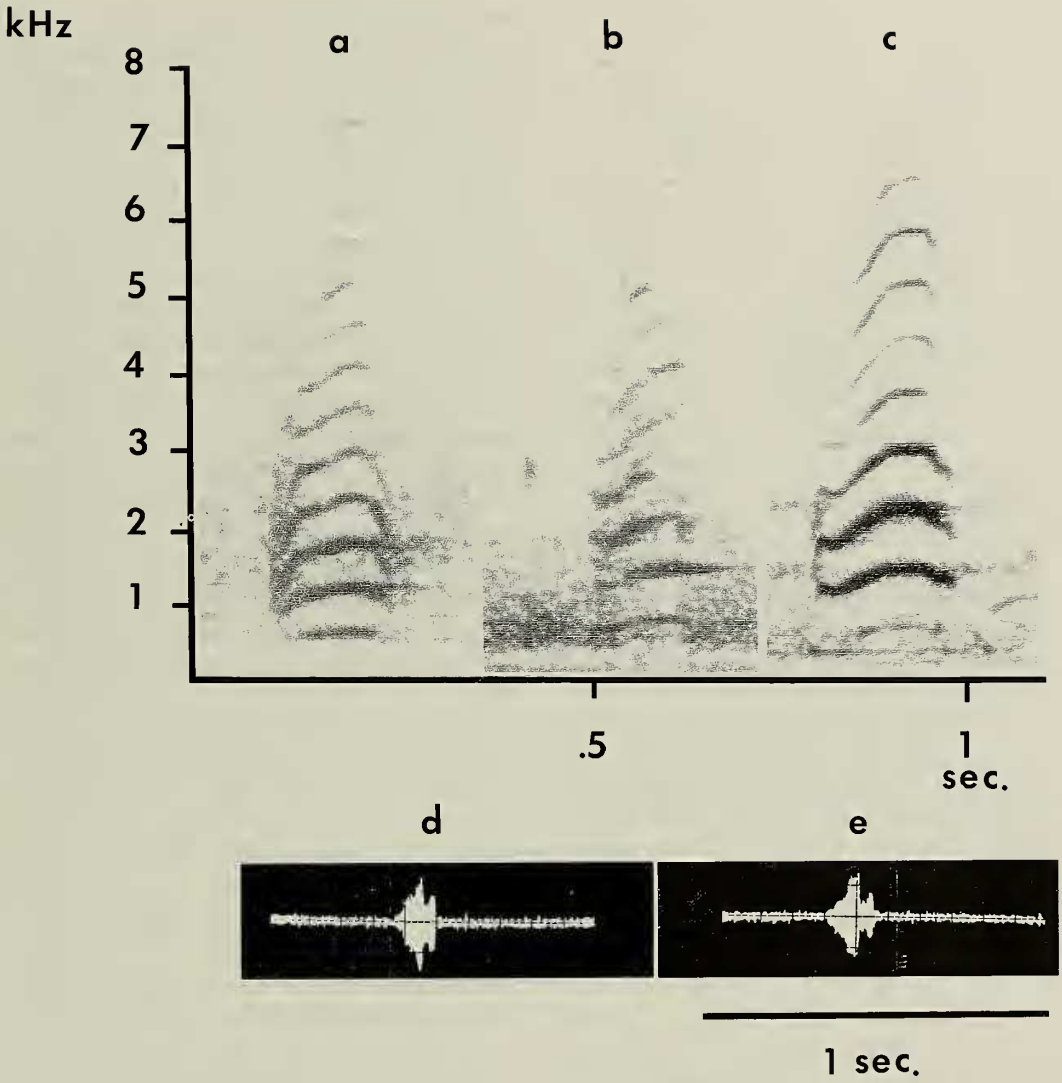

Fig. 1. Audiospectrograms of the advertisement call of *Eupsophus contulmoensis* (a), *E. insularis* (b), and *E. roseus* (c), band filter 45 Hz. Oscillograms of the advertisement call of *E. contulmoensis* (d) and *E. insularis* (e).

ing both of these species within this species group. The second group includes *E. vertebralis,* whose call contains 4–6 notes lasting 0.09 seconds. The advertisement call of *E. emiliopugini* shows that this species emits two notes lasting 0.20 seconds. Based on this information, we consider that *E. emiliopugini* should be grouped with *E. vertebralis,* rather than the *roseus* group.

Both species groups (i.e., *roseus* and *vertebralis*) are also supported when spectral parameters of the calls are considered. Members of the *vertebralis* group show lower mean values of dominant frequencies (1024–1350 Hz) than do members of the *roseus* group (1550–1900 Hz) (Table 1). On the other hand, members of the *vertebralis* group exhibit marked pulsed calls (11–34

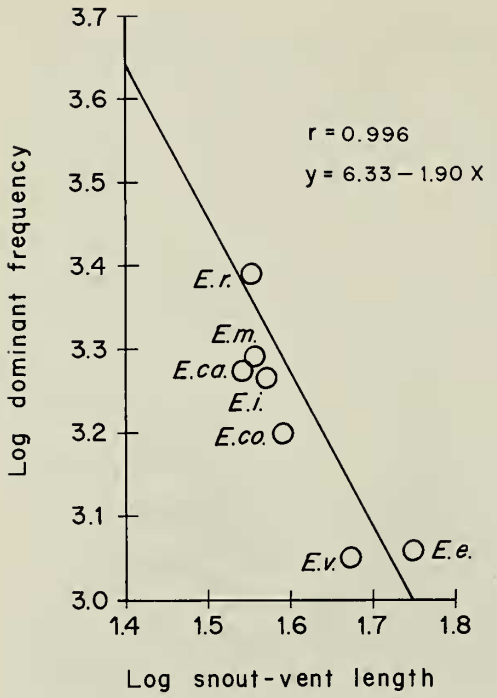

Fig. 2. Relation between mean snout–vent length and mean dominant frequency of the calls of *Eupsophus* species. Snout–vent length measurements correspond to samples of males from localities where recordings were made. Abbreviations (sample size in parentheses): E.r., *Eupsophus roseus* (12), E.m., *Eupsophus migueli* (7), E.ca., *E. calcaratus* (6), E.i., *E. insularis* (5), E.co., *E. contulmoensis* (7), E.v., *E. vertebralis* (14), E.e., *E. emiliopugini* (17).

pulses per note), while the *roseus* group species show calls poor in pulsations.

As indicated earlier, different authors have suggested that calls of frogs are useful in revealing systematic and evolutionary relationships. Temporal and spectral parameters of the advertisement calls of *Eupsophus* species may also reveal information concerning the evolutionary history of these species. Spectral components of the call (dominant frequencies) show wide overlap within *Eupsophus* (Table 1). The frequency range (700–2900 Hz) at which these species call is also shared by other frogs of the temperate *Nothofagus* forest (Penna & Veloso 1990), and thus cannot be related to particular physical features of the call sites of these frogs. When the mean dominant frequencies of *Eupsophus* species are compared, *E. emiliopugini* and *E. vertebralis* show lower values (1024–1350 Hz) than those presented by *E. contulmoensis, E. insularis, E. roseus, E. calcaratus* and *E. migueli* (1550–1900 Hz) (Table 1). The relationship between the mean dominant frequency of the call varies inversely with the logarithm of animal size ($r = -0.996$) (Fig. 2). The advertisement calls of *E. emiliopugini* and *E. vertebralis* have been described as conspicuously pulsed (Formas 1989). The former species emits 25.45 (17–34) pulses per note and the latter 15.90 (11–23) pulses per note. In contrast, calls of *E. roseus, E. migueli, E. contulmoensis, E. insularis* and *E. calcaratus* are poor in pulsation; however the "B" call (or long call) of *E. migueli* is markedly pulsed; 6–9 pulses per note (Formas 1985).

Eupsophus species exhibit considerable variation in calls, especially in the number of notes and their durations. Extremes are the single note (short call) of *E. roseus, E. migueli, E. calcaratus, E. contulmoensis,* and *E. insularis* (0.10–0.20 seconds), and the complex trilled calls (long call) with more than 40 notes of *E. roseus* and *E. migueli* (3.3–4.4 seconds). This latter vocalization has been interpreted as a territorial call (Formas 1985, Penna & Veloso 1990). Between these extremes are the advertisement calls of *E. emiliopugini* (2 notes) and *E. vertebralis* (4–6 notes lasting 0.40 to 0.80 seconds). If the length of each individual note is considered, the individual notes of the call of *E. emiliopugini* lasts 0.20 (0.19–0.23) seconds in duration, a value similar to the single notes of the advertisement calls of *E. roseus, E. calcaratus, E. migueli, E. contolmoensis,* and *E. insularis* ($\bar{X} = 0.18$; range 0.14–0.26 seconds). On the other hand, individual notes of the advertisement call of *E. vertebralis* have a duration ($\bar{X} = 0.089$; range 0.062–0.187) similar to the individual notes of the long call of *E. migueli* ($\bar{X} =$

0.083; range 0.051–0.092). In spite of this similarity, both calls are different in the number of pulses per note ($\bar{X}$ = 15.90 in *E. vertebralis;* $\bar{X}$ = 7.5 in *E. migueli*), number of notes per call (4–6 in *E. vertebralis*; more than 40 notes in *E. migueli*), and duration of the call ($\bar{X}$ = 0.6; range 0.4–0.8 seconds in *E. vertebralis* and $\bar{X}$ = 3.4; range 3.3–4.4 seconds in *E. migueli*).

Although members of the *roseus* group exhibit some similar patterns in advertisement calls, differences can be found when intervals among harmonics are examined. Calls of *E. contulmoensis* and *E. insularis* have harmonics at about 500 Hz intervals, while *E. calcaratus, E. migueli* and *E. roseus* show harmonics at about 1000 Hz intervals. A comparison among the calls of *E. roseus, E. contulmoensis* and *E. insularis* is shown in Fig. 1. In the case of the *vertebralis* species group, both members (*E. vertebralis* and *E. emiliopugini*) have harmonics at about 250 Hz intervals (Formas 1989).

Recognizing two species groups within the genus *Eupsophus* based on some temporal (call length and notes per call) and spectral (mean dominant frequencies and pulses) characteristics of advertisement calls agrees with the same proposal based on chromosomal data (Formas 1980). Members of the *vertebralis* group (*E. vertebralis* and *E. emiliopugini*) share a 28-chromosome karyotype (Formas 1991), while members of the *roseus* group have a 30-chromosome karyotype (Formas 1980, Iturra & Veloso 1981, 1989). Cuevas (personal communication) found that *E. insularis* and *E. contulmoensis* have a 30-chromosome karyotype, as occurs in *E. roseus, E. migueli,* and *E. calcaratus.*

Members of the *Eupsophus roseus* species group (*E. roseus, E. calcaratus, E. insularis, E. contulmoensis,* and *E. migueli*) are allopatric species; however, *E. roseus* and *E. migueli* are sympatric but not syntopic at the locality of Mehuín (39°26′S, 73°10′W) (Iturra & Veloso 1981). Members of the *E. vertebralis* species group (*E. vertebralis* and *E. emiliopugini*) are also allopatric. In spite of the fact that species of each group (*roseus* and *vertebralis*) are allopatric, it is possible to find members of the two different species groups in sympatry. For example, at the locality of Mehuín, *E. vertebralis* and *E. migueli* were observed while calling from cavities separated by two or three meters (Brieva, personal observation). A similar case of sympatry between members of the two different species group was observed at Cuesta de Soto (7 km by road from Valdivia city) between *E. roseus* and *E. vertebralis.* In three localities from southern Chile (La Picada, 42°07′S, 73°49′W; Río Rollizo, 41°27′S, 72°27′W; and Puntra, 42°07′S, 73°49′W) *E. emiliopugini* and *E. calcaratus* were observed calling from cavities separated by 25–50 cm (Formas 1989). Finally, *E. vertebralis* and *E. contulmoensis* were observed while calling from cavities separated by two to four meters at the locality of Contulmo (Natural Monument of Contulmo) (Formas, personal observation).

The four pairs of *Eupsophus* species recorded to occur in sympatry (*E. migueli* and *E. vertebralis*; *E. roseus* and *E. vertebralis*; *E. calcaratus* and *E. emiliopugini*; *E. vertebralis* and *E. contulmoensis*) are members of the two different species groups. These pairs of sympatric species exhibit a strong divergence among their advertisement calls. Passmore (1981) found that sympatric species of the African frog genus *Ptychadena* show remarkable differences in temporal and spectral components of their calls. In order to explain the origin of this phenomenon he used the notion of the Specific Mate Recognition System (SMRS) (Paterson 1978, 1982). According to this view, interspecific differences in signals do not agree with the traditional hypothesis that call divergence among closely related species develops due to selection for increasing reproductive isolation upon re-encounter of previously isolated populations (Dobzhansky 1970). Passmore's (1981) explanation for *Ptychadena* species is that the "pattern of vocal

divergence probably resulted from independent evolutionary histories, and concomitant with the development of their respective acoustical recognition mechanism, rather than from selective pressure for increasing reproductive isolation" (Penna & Veloso 1990).

In light of the SMRS concept we propose a hypothesis addressing the divergence of vocal patterns and the evolutionary history of the genus *Eupsophus*. As shown above (Table 1) the *vertebralis* group (*E. vertebralis* and *E. emiliopugini*) exhibits two different advertisement calls. Both members of this species group occur allopatrically (Formas 1989) and their karyotypes show differences in relation to chromosome pair No. 14 (telocentric in *E. vertebralis* and metacentric in *E. emiliopugini*; Formas 1991). From an evolutionary point of view, the genus *Eupsophus* has a long history, with early generic differentiation documented by fossil records dating back to the Oligocene (Schaeffer 1949, Baez & Gasparini 1979). Conditions for further acoustical and chromosomal diversification within these species existed during Pleistocene glaciations, during which reduced areas free of ice provided scattered refugia for the survival of flora and fauna (Vuilleumier 1968, Heusser 1974, Ashworth et al. 1991). Since the present geographical ranges of *E. vertebralis* and *E. emiliopugini* (especially the southermost part) are in an area that was affected by glaciations, we hypothesize that ice-free areas were the appropriate scenarios where acoustical and chromosomal differentiation took place. Frogs of the *roseus* group (*E. roseus, E. migueli, E. calcaratus, E. contulmoensis,* and *E. insularis*) are allopatric species sharing a similar one note advertisement call; however, *E. contulmoensis* and *E. insularis* have harmonics at about 500 Hz intervals while *E. roseus, E. migueli* and *E. calcaratus* have harmonics at about 1000 Hz intervals. Vocal differences among these allopatric species suggest that these species represent a recent stage of speciation. All members of the *roseus* group have the same chromosomal formula (2N = 30), although differences have been found among the karyotypes of some species. For example, the karyotypes of *E. roseus* and *E. migueli* (Iturra & Veloso 1989) exhibit marked similarities, but sex chromosomes differ. In these two species, the Y chromosome is a small metacentric one without paracentromeric heterochromatin, and corresponds to pair No. 14. In *E. roseus,* the sex chromosomes have the same metacentric morphology, but in *E. migueli* the X chromosome is telocentric. This example of chromosomal divergence between two members of the *roseus* group (which share the same temporal and spectral characteristic of the advertisement calls) suggests that chromosomal differentiation has played a more important role in the diversification of some members of the *roseus* species group than has the advertisement call. Finally, based on chromosome data, we suggest that vocal differences noted for *Eupsophus* species that occur in sympatry results from the presumed independent evolutionary histories of the taxa involved.

Acknowledgments

This study was supported by Fondo Nacional de Investigación Científica y Tecnológica (Proyecto 0050-89) and Dirección de Investigación y Desarrollo, Universidad Austral de Chile (Proyecto RS 89-06). We are grateful to Corporación Nacional Forestal (CONAF) for its hospitality and field facilities at Parque Nacional Contulmo and Isla Mocha. The audiospectrograms were done by Alfredo Brain, Instituto de Acústica, Universidad Austral de Chile. Mario Penna assisted us with the osilloscopic analyses. We wish to thank Carlos Jara for his useful comments on the manuscript. Raúl Arriagada aided with collections and tape recordings in the field. Our thanks to Ronald Heyer and two anonymous reviewers for their suggestions.

Literature Cited

Ashworth, A., V. Markgraf, & C. Villagrán. 1991. Late Quaternary climate of the Chilean Channels based on fossil pollen and beetle analyses with an analysis of the modern vegetation and pollen rain.—Journal of Quaternary Science 6:279–291.

Baez, A. M., & Z. B. Gasparini. 1979. The South American Herpetofauna: an evaluation of the fossil record. *In* W. E. Duellman, ed., The South American Herpetofauna: its origin, evolution and dispersal.—Museum of Natural History of Kansas Monographs 7:29–54.

Barrio, A. 1965. El género *Physalaemus* (Anura, Leptodactylidae) en la Argentina.—Physis 25:421–448.

Blair, W. F. 1958. Call structure and species group in U.S. tree frogs (*Hyla*).—Southwestern Naturalist 3:77–89.

Dobzhansky, T. 1970. Genetics of evolutionary process. Columbia Press, New York.

Formas, J. R. 1980. The chromosomes of *E. calcaratus* and the karyological evolution of the genus *Eupsophus* (Anura: Leptodactylidae).—Experientia 36:1163–1164.

———. 1985. The voices and relationships of the Chilean frogs *Eupsophus migueli* and *Eupsophus calcaratus* (Amphibia: Anura: Leptodactylidae).—Proceedings of the Biological Society of Washington 98:411–415.

———. 1989. A new species of *Eupsophus* (Amphibia: Anura: Leptodactylidae).—Proceedings of the Biological Society of Washington 102: 568–576.

———. 1991. The karyotypes of *Eupsophus emiliopugini* and *E. vertebralis* (Amphibia: Anura: Leptodactylidae).—Proceedings of the Biological Society of Washington 104:7–11.

———, & M. A. Vera. 1980. Reproductive patterns of *Eupsophus roseus* and *E. vittatus*.—Journal of Herpetology 14:11–14.

———, & M. I. Vera. 1982. The status of two Chilean frogs of the genus *Eupsophus*.—Proceedings of the Biological Society of Washington 95:594–601.

Heusser, C. J. 1974. Vegetation and climate of the Southern Chilean Lake District during and since the last glaciation.—Quaternary Sciences (N.Y.) 4:290–315.

Iturra, P., & A. Veloso. 1981. Evidence for heteromorphic sex chromosomes in male amphibians (Anura: Leptodactylidae).—Cytogenetic and Cell Genetics 31:108–110.

———, & ———. 1989. Further evidence for early sex chromosome differentiation of anuran species.—Genetica 78:25–31.

Kuramoto, M. 1974. Mating calls of Japanese tree frogs (Rhacophoridae).—Bulletin of the Fukuoka University, Faculty of Education 26:67–77.

Martin, W. F. 1972. Evolution of vocalization in the genus *Bufo*. Pp. 279–309 *in* W. F. Blair, ed., Evolution in the genus *Bufo*. University of Texas Press, Austin and London.

Mecham, T. S. 1971. Vocalization of the leopard frog, *Rana pipiens* and the related Mexican species.—Copeia 71:506–516.

Ortiz, J. C., H. Ibarra-Vidal, & J. R. Formas. 1989. A new species of *Eupsophus* (Anura: Leptodactylidae) from Contulmo, Nahuelbuta Range.—Proceedings of the Biological Study of Washington 102:1031–1035.

Passmore, N. I. 1981. The relevance of the specific mate recognition concept to anuran reproductive biology.—Monitore Zoologico Italiano (N.S.) Supplement XV:93–108.

Paterson, H. E. M. 1978. More evidence against speciation by reinforcement.—South African Journal of Science 74:369–371.

———. 1982. Perspective on speciation by reinforcement.—African Journal of Science 78:53–57.

Penna, M., & A. Veloso. 1990. Vocal diversity in frogs of the South American temperate forest.—Journal of Herpetology 24:23–33.

Ryan, M. J. 1988. Constraints and patterns in the evolution of anuran acoustic communication. Pp. 637–677 *in* B. Fritzsch, M. Ryan, W. Wilczynski, T. Hetherington, & W. Walkowiak, eds., The evolution of the Amphibian Auditory System. John Wiley, New York.

Schaeffer, B. 1949. Anurans from the early Tertiary of Patagonia.—Bulletin of American Museum of Natural History 93:41–68.

Schiøtz, A. 1973. Evolution of anuran mating calls, ecological aspects. Pp. 311–319 *in* J. L. Vial, ed., Evolutionary biology of the Anurans. University of Missouri Press, Columbia.

Straughan, I. R., & R. Heyer. 1976. A functional analysis of the mating calls of the neotropical frog genera of the *Leptodactylus* complex (Amphibia, Leptodactylidae).—Papéis Avulsos de Zoologia 29:221–245.

Vuilleumier, F. 1968. Origin of frogs of Patagonian forests.—Nature 219:87–89.

Instituto de Zoología, Universidad Austral de Chile, Casilla 567, Valdivia, Chile.